11 P.C. Panda, M.V. Shanka, M. Sceenivasa, I.A.S. Murti and I. Amla, Ind. J. exp. Biol. *6*, 569 (1975).
12 O. Bassir and T.C. Alozie, Toxicon, in the press.
13 R. Schoental, A. Rev. Pharmac. *7*, 343 (1967).
14 V.W. Chaffee, G.T. Edds, J.A. Himes and F.C. Neal, Am. J. vet. Res. *30*, 1737 (1969).
15 O. Bassir and E.A. Bababunmi, West Afr. J. Biol. appl. Chem. *12*, 28 (1969).
16 O. Bassir and E.A. Bababunmi, Br. J. exp. Path. *51*, 483 (1970).
17 G.R. Kerr, Physiol. Rev. *52*, 415 (1972).
18 O.L. Shotwell, C.W. Hesseltine, R.D. Stubblefield and W.G. Sorensen, Appl. Microbiol. *14*, 425 (1966).
19 G.C. Llewellyn, W.W. Carlton, J.E. Robbers and W.G. Hansen, Devs ind. Microbiol. *14*, 325 (1973).
20 J.V. Rodricks and L. Stoloff, J. Ass. off. agric. Chem. *53*, 92 (1970).
21 P.A. Owren, Lancet *2*, 754 (1959).
22 P.B. Hamilton, W.T. Tung, T.R. Harris, J.H. Gainer and W.E. Donaldson, Poult. Sci. *51*, 165 (1972).
23 H.T. Tung, W.E. Donaldson and P.B. Hamilton, Tox. appl. Pharmac. *22*, 97 (1972).
24 F.O. Osiyemi, P. Thesis, Ibadan University, Nigeria 1968.
25 T.V. Madhavan and G. Gopalan, Ind. J. med. Res. *53*, 984 (1965).

Activation by S-adenosylhomocysteine of norepinephrine and serotonin in vitro uptake in synaptosomal preparations from rat brain[1]

P. Fonlupt, M. Roche, L. Cronenberger and H. Pacheco

Laboratoire de Chimie Biologique and Laboratoire de Physiologie et Pharmacodynamie, Bâtiment 406, Institut National des Sciences Appliquées, 20, avenue Albert Einstein, F-69621 Villeurbanne Cédex (France), 9 October 1978

Summary. S-Adenosylhomocysteine (10^{-7}–10^{-5} M) activated norepinephrine (NE) and serotonin (5HT) in vitro uptake in synaptosomal preparations from rat brain, but did not affect dopamine (DA) uptake. When administered to rats (7 mg/kg i.p.), it had the same effect on in vitro NE and 5HT uptake. It did not affect NE and 5HT release.

S-Adenosylhomocysteine (SAH) is an inhibitor in numerous methylations[2], particularly methylations of nucleic acids and neurotransmitters[3]. Some studies have been performed relating to a possible antitumoral activity[4]. Recently we have discovered that SAH is sleep-inductive and anticonvulsant (electric shock) with rabbit, rat and cat (administered i.v., i.p. or p.o. in 1–10 mg/kg-doses)[5]. This result led us to consider the action of SAH on neurotransmitters uptake and release in synaptosomal preparations from rat brain.

Methods. The method of Snyder and Coyle[6] was used to measure dopamine (DA), norepinephrine (NE) and 5 hydroxytryptamine (5HT) uptake on a crude synaptosomal fraction, obtained from homogenates of part of male Wistar rat (150–200 g) brain. The brain was dissected out according to the method prescribed by Glowinski and Iversen[7]. After homogenization in a 0.32-M sucrose solution and centrifugation (1000× g), 0.3 ml of the synaptosomal suspension were incubated in 3 ml of Krebs Henseleit medium ($CaCl_2$ concentration 1.13 mM) containing ascorbic acid 1 mM, glucose 10 mM and nialamide $1.25 \cdot 10^{-5}$ M. The suspension was incubated for 10 min at 37 °C, before adding 0.2 ml of Krebs solution containing the tritiated neurotransmitters (0.2 µM), and for 5 min after addition; then it was chilled and centrifuged for 20 min (20,000× g at 4 °C). After rinsing twice the pellet with 3 ml of NaCl 9‰, and homogenizing in absolute alcohol, the radioactivity taken in synaptosomes was measured with liquid scintillation counting.

Release evaluation was achieved according to the method of Mulder et al.[8]. Synaptosomes (corresponding to about 50 mg fresh tissue), charged for 20 min with tritiated neurotransmitter, were laid on a Sephadex G 25 bed contained in a syringe (diameter 0.6 cm× 10 cm). After 35 min perfusion (0.25 ml/min), Krebs medium was replaced by identical medium containing SAH, then, 20 min later, for NE and 5HT tests, KCl concentration was

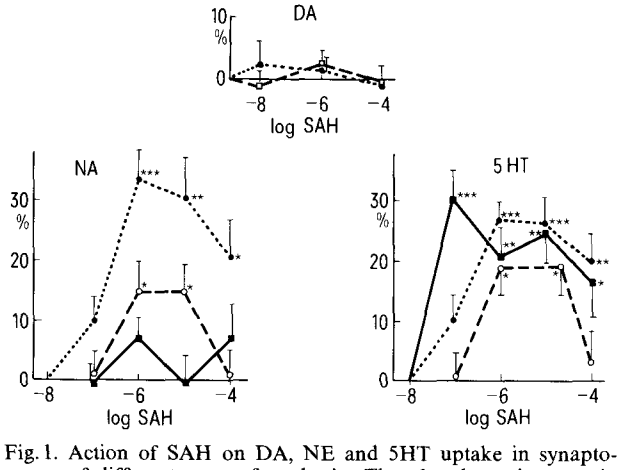

Fig. 1. Action of SAH on DA, NE and 5HT uptake in synaptosomes of different parts of rat brain. The plot shows increase in DA, NA, 5HT uptake in the presence of SAH, in percent of control values. Each point is the average of 8–12 determinations; statistical meaning of the difference between test and control sample is determined with t-test (*$p<0.05$; **$p<0.01$; ***$p<0.001$). ●···●, cortex; ○---○, midbrain + hypothalamus; ■——■, brainstem; □---□, corpora striata.

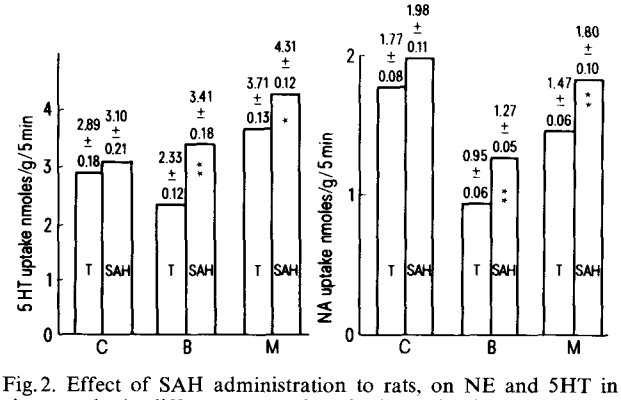

Fig. 2. Effect of SAH administration to rats, on NE and 5HT in vitro uptake in different parts of rat brain. Animals receive either SAH (7 mg/kg i.p.), or saline solution 1 h prior to sacrifice. The rest of the procedure is as before. Each value is the average of 7 determinations±SEM. Statistical meaning is determined with t-test (*$p < 0.05$; **$p < 0.01$). C: cortex + corpora striata; B: brainstem; M: midbrain + hypothalamus.

increased from 5 to 55 mM (release stimulation). Radioactive products used were D,L-norepinephrine ^{3}H-7, 8-20 Ci/mmole, serotonin ^{14}C-1 40 mCi/mmole, and dopamine ^{3}H 1-2, 15-20 Ci/mmole (CEA Saclay).

Results. With concentrations higher than 10^{-8} M, SAH increased NE and 5HT uptake in the 3 parts of the brain, but did not affect DA uptake (figure 1). Increase was significant in cortex (32%) and brainstem (17%) for NE and in cortex (25%), midbrain (28%) and brainstem (18%) for 5HT. SAH (10^{-6} or 10^{-5} M) did not alter DA, NE and 5HT release, either spontaneous or induced by KCl.

Administered in vivo to the animal 1 h prior to sacrifice (7 mg/kg i.p.) it induced in vitro uptake increase for NE and 5HT, significant only in brainstem and midbrain (figure 2), but did not alter DA uptake.

Discussion. The above results are to be compared with the action of 2 drugs acting on the central nervous system: a) Diphenylhydantoïne, an anticonvulsant product, induces in vitro NE uptake increase in the whole brain (NE 10^{-6} M)[9], but its effect is clear with a concentration 10 times higher than concentration of SAH. An increase of NE and 5HT uptake, when discharging is generalized, might explain the anticonvulsant effect of both drugs. b) Methionine sulfoximine, a convulsant product[10], lowers SAH level in different parts of rat brain[11].

Therefore SAH seems to be involved in the regulation of the metabolism of 2 neurotransmitters NE and 5HT. The 1st point of impact of SAH might be located at the level of catechol-O-methyltransferase; it is possible that SAH inhibits the formation of uptake-inhibiting methylated products. This mechanism seems to be rather unlikely, as, in our experimental conditions, few methylated products are formed, and the main product, normetanephrine, is a very weak uptake inhibitor[12]. Administration of SAH in vivo and observation of the effect in vitro seems to show a strong affinity of SAH for NE and 5HT uptake system; thus a direct action of SAH on uptake mechanism at the membrane level is more credible.

1 Work achieved within the frame of the research contract institut Mérieux., Institut National des Sciences Appliquées.
2 S.K. Shapiro and F. Schlenk, ed., Transmethylation and methionine Biosynthesis. University Chicago Press, Chicago, Ill. 1965.
3 R.J. Baldessarini, Int. Rev. Neurobiol. *18*, 41 (1975).
4 C.D. Chang and K. Coward, Molec. Pharmac. *11*, 701 (1975).
5 P. Fonlupt, M. Roche, L. Cronenberger and H. Pacheco, French patent 28 avril 1978, Institut Mérieux (n° 78 12711).
6 S.H. Snyder and J.T. Coyle, J. Pharmac. exp. Ther. *165*, 78 (1969).
7 J. Glowinski and L.L. Iversen, J. Neurochem. *13*, 655 (1966).
8 A.H. Mulder, W.B. Vandenberg and J.C. Stoof, Brain Res. *99*, 419 (1975).
9 M.G. Hadfield, Arch. Neurol. *26*, 78 (1972).
10 A. Lodin and J. Kolovsek, Physiol. bohemosl. *7*, 84 (1958).
11 R.A. Schatz, C.R. Vunnam and O.Z. Sellinger, Neurochem. Res. *2*, 27 (1977).
12 R.A. Maxwell, R.M. Ferris and J.E. Burcsu, in: The mechanism of neuronal and extraneuronal transport of catecholamines, p. 95. Ed. D.M. Paton. Rauer Press, New York 1976.

Possible mechanism of rubidium-induced hyperactivity in the rat

C.P. Chow[1] and H.H. Cornish

Interdepartmental Toxicology Program, The University of Michigan, Ann Arbor (Michigan 48109, USA), 26 October 1978

Summary. This study suggests that replacement of intracellular potassium by rubidium ions might lower the resting membrane potential. Thus rubidium-treated rats were more responsive to depolarizing influences and generated more cyclic AMP in the brainstem and consequently the behavioral changes.

Meltzer and associates[2] reported that rubidium injection increased the prevalent frequency of the electroencephalogram in monkey and altered the behavior in the direction of increased activity. They further suggested that rubidium, like lithium, might have therapeutic application in the affective disorders. Stolk et al.[3] reported that treatment of rats for 10 days with rubidium caused an increase in the rate of disappearance of norepinephrine in the brainstem after the biosynthesis of norepinephrine was inhibited, suggesting the antidepressant potential of rubidium. The antidepressant properties of rubidium in humans is being investigated in the metabolic wards of several countries[4,5]. Johnson[6] demonstrated that rubidium-treated rats recorded an increase in both the vertical rearing and horizontal locomotor activity. The objective of this investigation was to gain some insights into the mechanism of rubidium-induced hyperactivity in the rat.

Methods. Male Sprague-Dawley rats with an average b. wt of 150 g were purchased from Spartan Research Animals, Inc., Haslett, Michigan. Upon arrival, rats were randomly paired and housed in stainless steel cages. The animal room was illuminated from 08.00 to 20.00 h, and the thermostat was set at 25 °C. Rats were allowed a week to acclimate to the laboratory environment, and food was available ad libitum. Experimental animals were given 50 mM rubidium chloride in their drinking water while control rats received equimolar sodium chloride solution. Motor activity was measured on an activity platform (Lafayette Instrument Company) each day at 16.00 h. Only 1 control and 1 experimental rat were tested each day. Animals were taken to a dark room and acclimated for 30 min in solitude under red light. The motor activity for the next 30 min were then measured. The sensitivity of the instrument was adjusted so that only large body movements of the rat were recorded. An electric timer was used to terminate the experiment at the end of 30 min.

After ingestion of rubidium chloride solution for 4 weeks, each rat was acclimated to the microwave oven for 3 consecutive days to minimize stress prior to sacrifice. At 14.00 h on the following day, 1 control and 1 experimental rat were subjected to microwave irradiation individually, as described by Schmidt et al.[7,8]. Animals were then decapitated and the heads were chilled in ice. The brain was

Ingestion of 50 mM rubidium chloride in the drinking water for 1 month on brain potassium concentration* in the rat

	Control	Experimental
Rubidium	0.2±0.1	32.5±1.9
Potassium	118.8±4.4	84.4±3.3
Rubidium+potassium	119.0	116.9

*Values, expressed as meq/kg wet wt, represent the mean±SE of 5-8 animals.